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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 10/553,307 | 08/07/2006 | John C. Gebler | 64254(49991) | 1965 |

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| EXAMINER | |
|---|---|
| XU, XIAOYUN | |
| ART UNIT | PAPER NUMBER |
| 1777 | |

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|---|---|
| 12/14/2010 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

| *Office Action Summary* | **Application No.** | **Applicant(s)** | |
|---|---|---|---|
| | 10/553,307 | GEBLER ET AL. | |
| | **Examiner** | **Art Unit** | |
| | ROBERT XU | 1777 | |

***-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --***

## Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

## Status

1)☒ Responsive to communication(s) filed on *09 December 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

## Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,51,56,57,65,73,77,79,83,88 and 99-102* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

## Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

## Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Continuation of Disposition of Claims: Claims pending in the application are 1,2,4-13,15-18,48,49,51,52,56-60,65,66,70-73,77,79,83-88,90,91,93 and 99-102.
Continuation of Disposition of Claims: Claims withdrawn from consideration are 4-13,15-18,48,49,52,58-60,66,70-72,84-87,90,91 and 93.

## DETAILED ACTION

1. Request for reconsideration of the application filed on 12/09/2010, is acknowledged. No amendment was made to the claims. Claims 1, 2, 4-13,15-18, 48, 49, 51, 52, 56-60, 65, 66, 70-73, 77, 79, 83-88, 90, 91, 93 and 99-102 are pending, of which, claims 4-13,15-18,48,49,52,58-60,66,70-72,84-87,90,91 and 93 are withdrawn, claims 1, 2, 51, 56, 57, 65, 73, 77, 79, 83, 88, and 99-102 are considered on merits.

2. In response to reconsideration, the examiner maintains rejections over prior art established in the previous Office action.

### *Claim Rejections - 35 USC § 103*

3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

4. **Claims 1, 2, 51, 56, 57, 65, 73, 77, 79, 83, 88, 99, 101 and 102** are rejected under 35 U.S.C. 103(a) as being unpatentable over Huang et al (Analytical Biochemistry, 1999, IDS) (Huang) and as evidenced by Imaizumi et al. (Journal of Physical Chemistry, 1995) (Imaizumi) and Xu et al. (Tetrahedron Letters, 2001) (Xu).

In regard to Claim 1, Huang teaches a method of preparing a sample for mass spectrometry analysis. The method comprises:

a) obtaining a sample comprising an analyte (peptide from tryptic digested protein), the analyte comprises an exposed group ($NH_2$-terminus) (see page 307, right col. 2nd paragraph); and

b) reacting the analyte (peptide) with a triarylphosphonium labeling reagent (Tris(trimethoxyphenyl)phosphonium (TMPP) reagents) having a reactive group (acetyl-O-succinimide (AcOSu)) capable of reacting with the exposed group ($NH_2$-terminus) to form a triarylphosphonium-linked analyte (see page 307, right col. 3rd paragraph).

wherein the labeling reagent has a structure according to the formula

$[Ar_3P^+R]X^-$,

wherein

each Ar is an aryl group (methoxyphenyl) (see Scheme 1);

P is a phosphorous atom (see Scheme 1);

R is a reactive group comprising a functional group (AcOSu) that react with the exposed group ($NH_2$-terminus) to form a covalent bond thereby forming triarylphosphonium-linked analytes (TMPP-Ac-peptide) (see Scheme 1); and
$X^-$ is a negatively-charged counter ion ($Br^-$) (see Scheme 1).

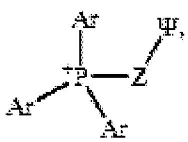
Huang does not teach that the $Ar_3P$ group is selected from the group consisting of unsubstituted naphthyldiphenylphosphine, dinaphthylphenylphosphine, trinaphthylphosphine, 9- anthryldiphenylphosphine, 9-anthryldinaphthylphosphine, diphenylpyrenylphosphine, dinaphthylpyrenylphosphine. Huang teaches that $Ar_3P$ is Tris(trimethoxyphenyl)phospine. The positive charge of Tris(trimethoxyphenyl)phospine is stabilized by the electron-donation from methoxyl substitute through the phenyl. Naphthyl, anthryl and pyrenyl have fused 2, 3, and 4 phenyl rings. They are known as fluorophores and have large conjugate orbital that can share electrons as evidenced by Imaizumi (see page 3810) and Xu (see page 9250). The conjugated system results in a general delocalization of the electrons across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the molecule. At time of the invention it would have been obvious to one of ordinary skill in the art to substitute trimethoxyphenyl with naphthyl, anthryl or pyrenyl in Huang's method in order to stabilize the phosphine by delocalizing the positive charge of phosphine. One would be motivated to do so because conjugated system of naphthyl, anthryl or pyrenyl would result in a general delocalization of the positive charge of phosphine across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the phosphine. The effect of the positive charge delocalization is similar to the effect of electron-donation. Trimethoxyphenyl (MW 168) is just as bulky as naphthyl (MW 128). Thus trimethoxyphenyl (MW 168) and naphthyl (MW 128) would be expected to have similar effect of a hindrance in the reactivity of these reagents with the analyte.

In regard to Claim 2, Huang teaches that the method comprising the further step of obtaining the triarylphosphonium labeling reagent having a reactive group (see page 307, left col. 3rd paragraph).

In regard to Claim 51, Huang teaches that the exposed group of the analyte (N-terminal group of peptide) is electrophilic and the reactive functional group (O-succinimide (OSu)) is nucleophilic (see scheme 1).

In regard to Claim 56, Huang teaches that $X^-$ is a halide ($Br^-$) (see scheme 1).

In regard to Claim 65, Huang teaches that the labeling reagent has the following structure:



wherein

each Ar is aryl group (methoxyphenyl) (see scheme 1);

P is a phosphorous atom (see scheme 1);

Z is a linking group (Ac) (see scheme 1); and

Ψ is a reactive functional group (OSu) (see scheme 1).

As has been discussed in regard to Claim 1 above, Huang does not teach that the $Ar_3P$ group is selected from the group consisting of unsubstituted naphthyldiphenylphosphine, dinaphthylphenylphosphine, trinaphthylphosphine, 9-anthryldiphenylphosphine, 9-anthryldinaphthylphosphine, diphenylpyrenylphosphine, dinaphthylpyrenylphosphine. Huang teaches that the $Ar_3P$ is Tris(trimethoxyphenyl)phospine. The positive charge of Tris(trimethoxyphenyl)phospine is stabilized by the  electron donated from trimethoxyl substitute through the phenyl. Naphthyl, anthryl and pyrenyl have fused 2, 3, and 4 phenyl rings. They are known as fluorophores and have large conjugate orbital that can share electrons as evidenced by Imaizumi and Xu. The conjugated system results in a general delocalization of the electrons across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the molecule.  At time of the invention it would have been obvious to one of ordinary skill in the art to substitute trimethoxyphenyl with naphthyl, anthryl or pyrenyl in Huang's method in order to stabilize the phosphine by delocalizing the positive charge of phosphine.  One would be

motivated to do so because conjugated system of naphthyl, anthryl or pyrenyl would result in a general delocalization of the positive charge of phosphine across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the phosphine. The effect of the positive charge delocalization is similar to the effect of electron-donation. Trimethoxyphenyl (MW 168) is just as bulky as naphthyl (MW 128). N-Tris(2,4,6-trimethoxyphenyl)phosphine (TMPP) has three methoxy groups attached to a phenyl ring, of which, two methoxyl groups are closer to the positively charged phosphine center. Naphthyl has fused phenyl ring to the original phenyl ring, which is further away from the positively charge phosphine center. Thus, 2,4,6-trimethoxyphenyl should have more steric hindrance than naphthyl to phosphine center. Thus trimethoxyphenyl (MW 168) and naphthyl (MW 128) would be expected to have similar effect of a hindrance in the reactivity of these reagents with the analyte.

In regard to Claim 73, Huang teaches that Ψ group is an isocynate (OSu) (see scheme 1).

In regard to Claim 77, Huang teaches that Ψ group is an aryl halide ($SC_6F_5$) (see scheme 1).

In regard to Claim 79, Huang teaches that Z has 3 nonhydrogen atoms selected from group consisting of C, N, O and S, and the longest liner segment contains 2 nonhydrogen atoms (see scheme 1).

In regard to Claim 83, Huang teaches that the analyte is a peptide (see scheme 1, page 307).

In regard to Claims 88 and 99, Huang does not specifically teach that the sample is a biological tissue. It is well known that proteins can be contained in biological tissue. Huang teaches that the resultant derivatized peptide mixture are subsequently analyzed by MALDI-PSD-MS using 0.5- to 1-pmol aliquots, giving rise to product ion spectra that are easily interpretable. As there is no need for material transfer and change of buffer media, the tandem enzymatic-chemical reaction/MS analysis process is usually carried out with very high throughput (see abstract). At the time of the invention it would have been obvious to one of ordinary skill in the art to analyzing a biological tissue that

contains proteins or peptides, in order to detect pmol sensitivity of the analyte in a biological tissue in a high throughput mode.

In regard to claims 101and 102, Huang teaches that the resultant derivatized peptide mixture are subsequently analyzed by MALDI-PSD-MS using 0.5- to 1-pmol aliquots, giving rise to product ion spectra that are easily interpretable. As there is no need for material transfer and change of buffer media (see abstract). Thus, Huang teaches that no further cleaned-up or desalting are needed after labeling (derivatizing).

5. **Claims 1, 88, 99 and 100** are rejected under 35 U.S.C. 103(a) as being unpatentable over Leavens et al (Rapid Communications in Mass Spectrometry, 2002, IDS) (Leavens) and as evidenced by Imaizumi and Xu.

In regard to Claim 1, Leavens teaches a method of preparing a sample for mass spectrometry analysis. The method comprises:

a) obtaining a sample comprising an analyte (amines) (see Table 1), the analyte comprises an exposed group (amine group) (see page 439, right col. 1st paragraph); and

b) reacting the analyte (amines) with a triarylphosphonium labeling reagent (TMPP-reagents) having a reactive group (carboxylic group) capable of reacting with the exposed group (amine group) to form a triarylphosphonium-linked analyte (see Table 3, page 439, right col. 1st paragraph).

wherein the labeling reagent has a structure according to the formula

$[Ar_3P^+R]X^-$,

wherein

each Ar is an aryl group (methoxyphenyl) (see Scheme 1);

P is a phosphorous atom (see Scheme 1);

R is a reactive group comprising a functional group (carboxylic group) that react with the exposed group (NH2-terminus) to form a covalent bond thereby forming triarylphosphonium-linked analytes (TMPP-Ac-peptide) (see Scheme 1); and

$X^-$ is a negatively-charged counter ion ($Br^-$) (see Scheme 1).

Leavens does not teach that the $Ar_3P$ group is selected from the group consisting of unsubstituted naphthyldiphenylphosphine, dinaphthylphenylphosphine,

trinaphthylphosphine, 9- anthryldiphenylphosphine, 9-anthryldinaphthylphosphine, diphenylpyrenylphosphine, dinaphthylpyrenylphosphine. Leavens teaches that the $Ar_3P$ is Tris(trimethoxyphenyl)phospine. The positive charge of Tris(trimethoxyphenyl)phospine is stabilized by the electron donated from trimethoxyl substitute to the phenyl. Naphthyl, anthryl and pyrenyl have fused 2, 3, and 4 phenyl rings. They are known as fluorophores and have large conjugate orbital that can share electrons as evidenced by Imaizumi (see page 3810) and Xu (see page 9250). The conjugated system results in a general delocalization of the electrons across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the molecule. At time of the invention it would have been obvious to one of ordinary skill in the art to substitute trimethoxyphenyl with naphthyl, anthryl or pyrenyl in Leavens' method in order to stabilize the phosphine by delocalizing the positive charge of phosphine. One would be motivated to do so because conjugated system of naphthyl, anthryl or pyrenyl would result in a general delocalization of the positive charge of phosphine across all of the adjacent parallel aligned p-orbitals of the atoms, which increases stability and thereby lowers the overall energy of the phosphine. The effect of the positive charge delocalization is similar to the effect of electron-donation. Trimethoxyphenyl (MW 168) is just as bulky as naphthyl (MW 128). N-Tris(2,4,6-trimethoxyphenyl)phosphine (TMPP) has three methoxy groups attached to a phenyl ring, of which, two methoxyl groups are closer to the positively charged phosphine center. Naphthyl has fused phenyl ring to the original phenyl ring, which is further away from the positively charge phosphine center. Thus, 2,4,6-trimethoxyphenyl should have more steric hindrance than naphthyl to phosphine center. Thus trimethoxyphenyl (MW 168) and naphthyl (MW 128) would be expected to have similar effect of a hindrance in the reactivity of these reagents with the analyte.

In regard to Claims 88 and 99, Leavens teaches a method of preparing a sample for mass spectrometry analysis, comprising

a) obtaining a sample comprising an analyte (amines) having an exposed group (amine group) (see page 439, right col. 1$^{st}$ paragraph, Table 1); and

b) reacting the analyte (amines) with a triarylphosphonium labeling reagent (TMPP-reagents) having a reactive group (carboxylic group) capable of reacting with the exposed group (amine group) to form a triarylphosphonium-linked analyte (see Table 3, page 439, right col. 1st paragraph).

Leavens does not specifically teach that the sample is a biological tissue. It is well known that biological tissue contains amines as well as other molecules. At the time of the invention it would have been obvious to one of ordinary skill in the art to take the advantage of the labeling agent in analyzing a biological tissue that contains amines. One would be motivated to do so because the labeling reagent can specifically react with amines in a biological tissue and makes the specific detection of amines in a biological tissue much easier.

In regard to Claim 100, Leavens teaches that the analyte is a small molecule (amines) (see Table 1).

### ***Response to Arguments***

6. Applicant's arguments filed 12/09/2010 have been fully considered but they are not persuasive.

Applicant contends that one of ordinary skill in the art, upon reading of the use of proteins as a sample in Huang or amines as a sample in Leavens would have had no motivation and no reasonable expectation of success in using a biological tissue itself as a sample for Mass Spectrometry. Examiner respectfully disagrees. The method of Huang and Leavens significantly enhance the sensitivity of labeled analyte detection to pmol level. There is no reason why a routineer would not use the same method to detect labeled analytes in solution of biological tissue itself as a sample for mass spectrometry. The labeling compound specifically reacts with the analyte in the sample mixture.

Applicants note that steric bulk and molecular weight are different factors. Indeed, just because a molecule has a heavier element (such as oxygen) present in its formula, the steric geometry of the molecule may still be relatively small. In response, N-Tris(2,4,6-trimethoxyphenyl)phosphine (TMPP) has three methoxy groups attached to a

phenyl ring, of which, two methoxyl groups are closer to the positively charged phosphine center. Naphthyl has fused phenyl ring to the original phenyl ring, which is further away from the positively charge phosphine center. Thus, 2,4,6-trimethoxyphenyl should have more steric hindrance than naphthyl to phosphine center.

Applicant argues that nothing in Huang not Leavens describes this increased efficiency, particular with regard to the testing of a biological tissue. However, Huang teaches that the resultant derivatized peptide mixture are subsequently analyzed by MALDI-PSD-MS using 0.5- to 1-pmol aliquots, giving rise to product ion spectra that are easily interpretable. As there is no need for material transfer and change of buffer media, the tandem enzymatic-chemical reaction/MS analysis process is usually carried out with very high throughput (see abstract). Thus, TMPP-Ac derivatized peptides from biological tissue would have pmol sensitivity to give rise to product ion spectra that are easily interpretable and the efficiency is very high throughput.

## *Conclusion*

7. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROBERT XU whose telephone number is (571)270-5560. The examiner can normally be reached on Mon-Thur 7:30am-5:00pm, Fri 7:30am-4:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Vickie Kim can be reached on (571)272-0579. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

12/13/2010

/Yelena G. Gakh/
Primary Examiner, Art Unit 1777

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